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RESEARCH ARTICLE

ROLE OF PREVIOUSROTAVIRUS INFECTION AND ITS ASSOCIATION WITH IFN-A IN OCCURRENCE OF CELIAC DISEASE IN IRAQI PATIENTS.

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Abstract

Celiac disease(CD) is a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals, many environmental triggering factors are suggested to participate in its pathogenesis, Several studies were struggled to establish the association between some microbial infections and CD development, some of these studies were suggested an association between rotavirus infections and CD development, but this association still unconfirmed yet, so that, this study aims to evaluate the role of previousrotavirusinfections and its association with IFN-α in occurrence of celiac disease.Sample of 80 Iraqi celiac patients has been chosen from all suspected patients who attending to Al-Suder- Medical city during the period of April 2015 to November 2015, also 80 healthy individuals (control) were included in this study, all patients were undergo blood investigations for anti- rotavirus IgG ,AGA-IgA, AGA-IgG, tTG-IgA , tTG-IgG and IFN-α tests. Results showeda significant decrease in levels of anti-rotavirus IgG in celiac patients compared to healthy group ,$p < 0.05$, and a significant increasing in levels of IFN-α in celiac patients compared to healthy group ,$p < 0.05$, also there is significant negative correlation between IFN-α and anti-rotavirus IgG in celiac patients, $P < 0.01$.Significant correlation between anti-rotavirus IgG and AGA-IgA,$P< 0.01(P=0.004)$, also there is significant correlation between anti-rotavirus IgG and tTg-IgG,$P< 0.01(P=0.007)$. In other hand ,there is no significant correlation between anti-rotavirus IgG and tTg-IgA, $P > 0.01(P=0.702)$ and no significant correlation between anti-rotavirus IgG and AGA-IgG, $P > 0.01(P=0.154)$.

Introduction:-

Celiac disease (CD) is a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals(1). A complex disorders, with environmental and genetic factors contributing to celiac disease etiology (2). The main genetic influence on celiac disease is the HLA locus (3), Around 90–95% of celiac patients express HLA-DQ2 heterodimer while the remaining 5–10% patients express the HLA-DQ8 heterodimer (4). Infectious agents are considered as possible environmental factors that triggering autoimmune diseases, exposure to infectious agents has also been suggested as a factor causing tissue damage and inflammation, which could eventually contribute to reduced gluten tolerance in celiac disease(5), several studies have described a possible link between celiac disease onset in susceptible patients with diverse infectious agents, and several hypotheses about the possible pathogenic mechanisms behind these associations have been discussed in present decade, including antigenic mimicry or increased immune activation secondary to infection-mediated inflammation by induction of TNF-α, IFN-γ, INF-α and IL-15 (5).Association between rotavirus infection and celiac disease development is suggested (6), but even now, the association between celiac disease predisposition and

certain microbial infection suggested but not confirmed(2). Most people had an infection with rotavirus during childhood by 3 year of age, repeated infections are measurable by an increase in the specific antibodies level of rotavirus (7), a single infection with rotavirus is unlikely to be a sufficient cause of CD ,therefore an increased in frequency of rotavirus infections might contribute to the development of CD (6). So that, the objective of this study is to evaluate if there is an increase in anti-rotavirus IgG in celiac patients as an indicator of previous rotavirusinfections compared with healthy group, also to evaluate the correlation between anti-rotavirus IgG and levels of AGA, Ttg and IFN-α in celiac patients.

Materials and Methods:-

Eighty Iraqi CD patients have been chosen from all suspected patients who attending to Al-Suder- teaching hospital during the period of April 2015 to November 2015 were included in this study. The age of those patients ranged from 1 year to 45 years, all patients were undergo blood sample collection to investigate anti- rotavirus IgG, AGA-IgA, AGA-IgG, tTG-IgA , tTG-IgG and IFN-α tests , also 80 apparently healthy individuals from general population who had negative serological markers for celiac disease were included as control group.

Serological markers of CD (AGA-IgA, AGA-IgG, Anti-tTg-IgA and Anti-tTg-IgG) tests were done by ELIZA technic ,according to instructions of manufacture company (Aeskulisa/Germany), anti-rotavirus IgG test was done by ELIZA technic, according to instructions of manufacture company (Qayeebio/ China) and IFN-α test was done by ELIZA technic, according to instructions of manufacture company (Elabscience/China).Statistical analysis were done by SSPS (ver. 14).

Results and discussion:-

Anti-rotavirus IgG was assayed in 80 celiac patients and 80 apparently healthy individuals by ELIZA technic, Statistical parameters showed that mean of Anti rotavirus IgG values was 91.06 ng/ml with Sd= 59.58 for celiac patients and 128.62 ng/ml with Sd=108.22 for healthy individuals , analysis of the difference between two groups means using (t-test), showed a significant decrease of anti-rotavirus IgG values among celiac patients compared to healthy group, $P< 0.05$ ($p=0.007$), as shown in table (1).

The correlation between anti-rotavirus and any of CD serological markers(AGA-IgA, AGA-IgG, Anti-tTg-IgA and Anti-tTg-IgG) of 80 celiac patients has been obtained by using **Pearson** correlation test, analysis of data showed there is significant negative correlation between anti-rotavirus IgG and AGA-IgA with $r = -0.315$ at 99% confidence interval ,$P < 0.01$($P=0.004$), also there is significant negative correlation between anti-rotavirus IgG and tTg-IgG with $r = -0.300$ at 99% confidence interval ,$P < 0.01$($P=0.007$) ,as shown in table (2). In other hand ,there is no significant correlation between anti-rotavirus IgG and tTg-IgA, $P > 0.01$($P=0.702$) and no significant correlation between anti-rotavirus IgG and AGA-IgG, $P > 0.01$($P=0.154$), as shown in table (2).

Table 1: Anti rotavirusIgG levels in healthy and celiac patients.

Parameters Groups	N	Anti-Rotavirus IgG levels			t- value	P-value (2-tailed)	95% confidence interval of difference	
		Mean	Sd	Std.Error Mean			Lower	Upper
Patients	80	91.06 ng/ml	59.58	6.66	-2.72	0.007	-64.84	-10.28
Healthy	80	128.62 ng/ml	108.22	12.09				

Table 2: Correlation between anti-rotavirus IgG and serological markers of CD.

		AGA.IgA	AGA.IgG	tTg.IgA	tTg.IgG	Rotavirus.IgG
AGA.IgA	Pearson Correlation	1	.573**	.031	.498**	-.315**
	Sig. (2-tailed)		.000	.785	.000	.004
	N	80	80	80	80	80
AGA.IgG	Pearson Correlation	.573**	1	.082	.659**	-.161
	Sig. (2-tailed)	.000		.470	.000	.154
	N	80	80	80	80	80
tTg.IgA	Pearson Correlation	.031	.082	1	.050	-.043
	Sig. (2-tailed)	.785	.470		.659	.702
	N	80	80	80	80	80
tTg.IgG	Pearson Correlation	.498**	.659**	.050	1	-.300**
	Sig. (2-tailed)	.000	.000	.659		.007
	N	80	80	80	80	80
Rotavirus.IgG	Pearson Correlation	-.315**	-.161	-.043	-.300**	1
	Sig. (2-tailed)	.004	.154	.702	.007	
	N	80	80	80	80	160

**. Correlation is significant at the 0.01 level (2-tailed).

In general, these results are show that level of anti-rotavirus IgG less in patients with celiac disease than in healthy group, that reduce the hypothesis of that celiac disease might come from previous rotavirus infection and this may come in agreement with the results of other researcher, who showed that the prevalence of rotavirus infection has no statistically significant difference between adults who had tTg antibody positive and those who had tTg antibody negative (8).

Also , these results agree with the results that obtained by Al-Hilali, when he studded CD autoantibody in Iraqi children with rotavirus infection and showed that slight or no chance in agreement(using K test) of that children with rotavirus infection positive will develop celiac disease (9). Furthermore, other authors were also found that no significant difference in the cumulative incidence of CD when comparing incidence of CD in children that were administered a rotavirus vaccination compare those who were not administered the vaccination (10).

In other hand, the results of this study may or may not differ from the results that obtained by Stene and his coworkers ,when they showed that high frequency of rotavirus infection (measured by increased anti- rotavirus IgA or IgG titer) my increase the risk of celiac disease in childhood in genetically predisposed individuals (6), so that this results defer from the results of Stene, because Stene and his coworkers reported that the frequency of rotavirus infection lead to increase of anti- rotavirus IgA or IgG titer in celiac patients ,while, anti- rotavirus IgG has been reported significantly decreased in celiac patients compared to healthy in this study, but may agree with results of Stene and his coworkers in that frequency of rotavirus infection may lead to increase the production of IFN-α, that may involve in CD pathogenesis, whatever, even now, the association between CD predisposition and rotavirus suggested but not confirmed (2).

However, the significant difference of anti-rotavirus IgG between healthy and celiac patients and significant negative correlation between anti- rotavirus IgG and AGA-IgA/or tTg-IgG that obtained in this study suggested that celiac patients may have less chance to get rotavirus infection than other, because CD and its associated inflammation that lead to intestinal symptoms, villous atrophy, crypt hyperplasia(11), may change enterocytes receptors for rotavirus binding, and rotavirus was found to specifically bind to villus tip membranes, also specific host cell receptor expression may be important in rotavirus pathogenesis (12)**,** so that, CD may reduce rotavirus infection.

In other hand, CD may modify immune response that lead to decrease humoral response against rotavirus consequently reduce level of anti-rotavirus IgG , this hypothesis supported by the negative correlation between anti-rotavirus IgG and anti- gluten markers that observed in this study, and by the observation of Ouakaa-Kchaou and his

assistants, when they show that level of anti-HBs antibody is reported to be lower in untreated celiac patients compared with healthy controls (13).

Also other researcher studded the association between CD and five major infectious agents, including Epstein-Barr virus (EBV), Cytomegalovirus (CMV), rubella virus, Toxoplasmagondii and Treponemapallidum and demonstrated a lower prevalence of IgG antibodies in serum of CD patients compared to healthy persons, suggesting a probable protective role of CMV, EBV and rubella virus on development of CD (14), in fact this finding agree with the finding of this study ,because it showed decrease in the IgGAb against each microorganisms ,that imply CD modify immune response that lead to decrease humoral response against each microorganisms ,but not infection with each microorganisms have a productive from celiac disease development.

Correlation between anti-rotavirus IgG and INF-α in celiac patients:-

IFN-α and anti-rotavirus IgG were assayed in all celiac patients and healthy groups, the mean value of IFN-α in healthy group was 28.49 pg /ml ,while the mean value of IFN-α for celiac patients group was 69.25 pg/ml, statistical analysis of data by using t-test to compare difference between the two groups showed a significant increasing in levels of IFN-α in celiac patients compared to healthy group ,$p < 0.05$ ($p = 0.001$), as shown in table (3).While, statistical analysis by using Pearson correlation coefficient showed there is significant negative correlation between IFN-α and anti-rotavirus IgG in celiac patients at 99% confidence interval ,$P < 0.01$ ($P=0.008$), as shown in table (4).

Table 3: IFN-α values in healthy and celiac patients groups.

parameters Groups	N	Mean	Sd.	Std.Error	t- value	P-value	95%confidence interval of difference	
							Lower	Upper
Patients	80	69.25 pg/ml	95.03	10.62	3.75	0.001	19.3	62.2
Healthy	80	28.49 pg/ml	20.34	2.27				

Table 4: Correlation between anti-rotavirus IgG and INF-α in celiac patients.

		Rotavirus.IgG	IFN.Alpha
Rotavirus.IgG	Pearson Correlation	1	-.292**
	Sig. (2-tailed)		.008
	N	160	80
IFN.Alpha	Pearson Correlation	-.292**	1
	Sig. (2-tailed)	.008	
	N	80	80

**. Correlation is significant at the 0.01 level (2-tailed).

The results of this study mean that levels of anti-rotavirus IgG inversely associate with level of IFN-α in serum of celiac patients.

Type I IFNs can be secreted in the response to infection with viruses and intracellular bacteria, also enterovirus infections were shown to provoke the secretion of IFN-α in the intestine (15) , in other hand type I IFNs exert potent anti- viral and immune regulating activity promoting the differentiation and maintenance of T h1 cells (16) and this results in an up-regulation of IFN-γ and IL-15 production by DCs (17) , two pro-inflammatory cytokines that are known to be involved in the pathogenesis of CD (18).

However , viral infections, in particular enterovirus infections such as rotavirus infections, have been suggested to increase the incidence of coeliac disease(6, 19) and other autoimmune disorders such as type-1 diabetes (20,21). Because viral infections lead to type-I IFN production (20, 22), also, type-I IFNs could also be induced by bacterial

infections(23,24) and type-I IFNs have immunostimulatory properties (22), also IFN-α production would lead to a shift towards Th1 responses and reinstruct previously tolerogenic DCs to prime gluten-specific T cells and support inflammation instead of sustaining oral tolerance (25).

Morever,Th1 responses and inflammation that associated with local production of IFN-α, play an important role in the anti-viral immunity (26,27).

So that , IFN-α may elevated due to infection with other type of viruses or bacteria in celiac patients and its anti-viral activity lead to decrease the level of anti-rotavirus IgG, that explain the negative correlation between IFN-α and the specific Ab against rotavirus which obtained in this study. As a result, we assume that viral infections might trigger CD as a consequence of IFN-α induction ,but not by the infection per situ, because virtually every child experiences episodes of rotavirusgastroenteritis in the first three years of live (28,29),but not all develop CD. Hence, repeated rotavirus infection increase risk of CD (6), my due to the long duration of IFN-α induction or due to as yet undefined host factors that are not related to infections, or/and may due to other type of viruses ,but yet un defined .

Conclusion:-

1. There is no direct association between previous rotavirus infection and celiac disease development.
2. celiac disease reduce specific immune response against rotavirus.
3. IFN-α has a significant role in celiac disease development.

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